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(57) Abstract

The present invention relates to an immunogenic determinant. More particularly, it relates to a protein or a protein fragment for use in the diagnosis of Kaposi's sarcoma or human herpesvirus 8 (KSHV/HHV8) and to a diagnostic kit utilising the protein or protein fragment of the invention. The amino acid sequence of the carboxyterminal end of KSHV orf 65 is given below: Sequence 1: Amino acids 86 - 170 of KSHV orf 65. ADRVSAASAY DAGTFTVPSR PGPASGTTPG GQDSLGVSGS SITTLSSGPH SLSPASDILT TLSSTTETAA PAVADARKPP SGKKK.

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AN IMMUNOGENIC DETERMINANT FOR USE IN THE DIAGNOSIS OF KAPOSI'S SARCOMA

DESCRIPTION

The present invention relates to an immunogenic determinant. More particularly, it relates to a protein or a protein fragment for use in the diagnosis of Kaposi's sarcoma or human herpesvirus 8 (KSHV/HHV8) and to a diagnostic kit utilising the protein or protein fragment of the invention.

Kaposi's sarcoma - related herpesvirus (KSHV) or Human herpesvirus 8 (HHV 8) may be the postulated infectious cause of Kaposi's Sarcoma and is closely related to Epstein-Barr virus (HHV 5). Its prevalence in the general population is controversial. Antibodies to latent KSHV/HHV 8 antigen(s) are largely restricted to individuals with, and at risk for, KS. The antibody response to different EBV antigens (viral capsid, early, latent antigen) varies in individuals with acute, latent, or reactivated EBV infection.

Epidemiological evidence suggests that Kaposi's sarcoma in both HIV-uninfected and -infected individuals is caused by a transmissible agent: Among HIV-infected patients KS is much more common in gay men than in other HIV transmission groups, in particular haemophiliacs, transfusion recipients and intravenous drug users (IVDU). Transmission of the putative KS agent occurs independently of that of HIV and shows a marked geographic variation. Similarly, the incidence of KS outside HIV infection differs dramatically in different geographic areas: In Africa, it is higher in central and Eastern Africa than in West or South Africa, and in Europe, it is commoner in some Mediterranean countries.

The recently discovered Kaposi Sarcoma associated herpesvirus (KSHV), or human herpesvirus 8 (HHV8) is a γ_2-herpesvirus and closely related to EBV. KSHV/HHV 8 may be the infectious cause of KS, as it is consistently found in all forms of Kaposi's sarcoma, i.e. AIDS-related KS, African endemic KS, classic HIV-negative KS and KS in transplant patients. KSHV/HHV 8 genomes are present in endothelial and spindle cells, the histological hallmark of KS lesions. Detection of KSHV/HHV 8 in peripheral blood correlates with, and in asymptomatic HIV-infected individuals predicts

the development of, KS lesions. However, whether the distribution of KSHV/HHV 8 matches that expected for the putative KS agent, is still controversial: while several groups have not detected KSHV/HHV 8 by PCR in the peripheral blood of healthy blood donors, others have reported its presence in 9% of PBMC and lymphoid tissue from HIV-uninfected persons. Similarly, some, but not other groups have reported a high prevalence in semen samples from healthy donors. More recently, the first serological studies have been carried out using immunoblotting and immunofluorescence assays on B-cell lymphoma cell lines, either dually infected with KSHV/HHV 8 and EBV, (Moore, P.S., Gao,S.-J., Dominguez, G., *et al.* Primary characterization of a herpesvirus agent associated with Kaposi's sarcoma. *J.Virol.*1996; 70: 549 - 558; Miller, G., Rigsby,M.O., Heston,L., *et al.* Antibodies to butyrate-inducible antigens of Kaposi's sarcoma - associated herpesvirus in patients with HIV-1 infection. *N.Engl.J.Med.* 1996; 334:1292 - 1297; Gao, S.J., Kingsley,L., Hoover, D.R. et al. Seroconversion of antibodies to Kaposi's sarcoma-associated herpesvirus - related latent nuclear antigens before the development of Kaposi's sarcoma. *N.Engl.J.Med.*1996; 335: 233 - 241) or only infected with KSHV/HHV 8 (Gao, S.J., Kingsley, L., Li, M. et al. Seroprevalence of KSHV antibodies among North Americans, Italians, and Ugandans with and without Kaposi's sarcoma. *Nature Medicine* 1996; 2: 925 - 928 (Kedes, D.H., Operskalski,E., Busch, M. et al. The seroprevalence of human herpesvirus 8 (HHV 8): Distribution of infection in Kaposi's sarcoma risk groups and evidence for sexual transmission. *Nature Medicine* 1996; 2: in press). Antibodies to latent KSHV/HHV 8 antigen(s) were found in the vast majority of KS patients, but not, or only rarely, in HIV-infected IVDU, haemophiliacs, or healthy blood donors. Seroconversion to latent nuclear antigens can occur years prior to KS onset and is strongly predictive of subsequent disease (Gao, S.J., Kingsley,L., Hoover, D.R. et al. Seroconversion of antibodies to Kaposi's sarcoma-associated herpesvirus - related latent nuclear antigens before the development of Kaposi's sarcoma. *N.Engl.J.Med.*1996; 335: 233 - 241; Gao, S.J., Kingsley, L., Li, M. et al.

Seroprevalence of KSHV antibodies among North Americans, Italians, and Ugandans with and without Kaposi's sarcoma. *Nature Medicine* 1996; 2: 925 - 928). The antibody response to different antigen complexes (latent, capsid, early antigen) of the closely related EBV varies in acutely vs. chronically infected individuals or immuosuppressed patients with reactivated EBV infection (Crawford, D.H. Epstein-Barr Virus in: Clinical Virology, Zuckerman, A.J., Banatvala, J.E., Pattison, J.R. eds., John Wiley & Sons, New York, 1995; pp. 109 - 14; Horneff, M.W., Bein, G., Fricke, L., *et al.* Coincidence of Epstein-Barr virus reactivation, cytomegalovirus infection, and rejection episodes in renal transplant recipients. *Transplantation* 1995; 60: 474 - 480). Several potentially immunoreactive lytic-cycle proteins of KSHV/HHV 8, including the major capsid protein (MCP), are highly homologous to their EBV counterparts (Moore, P.S., Gao,S.-J., Dominguez, G., *et al.* Primary characterization of a herpesvirus agent associated with Kaposi's sarcoma. *J.Virol.*1996; 70: 549 - 558) and are thus unlikely to provide specific serological antigens.

In seeking an immunogenic determinant, recombinant proteins were generated in E. Coli from a number of orf encoded genes of KSHV/HHV8.

Particularly favourable results were obtained with a recombinant protein to orf 65 - a lytic cycle/capsid related structural protein. This way particularly surprising given that KSHV/HHV8 homologues of at least two immunoreactive EBV proteins orf 52 and orf 29b had failed to react (as recombinant proteins) with sera from patients with Kaposi's Sarcoma.

More particularly the applicant has determined that the immunogenic region of the orf 65 protein was to be found at or within the carboxyterminal end - ie about the last 80 amino acids.

The amino acid sequence of the carboxyterminal end of KSHV orf 65 is given below:

Sequence 1: Amino acids 86 - 170 of KSHV orf 65

ADRVSAASAY DAGTTTVPSR PGPASGTTPG GQDSLGVSGS SITTLSSGPH SLSPASDILT TLSSTTETAA PAVADARKPP SGKKK

It is an aim of the present invention to identify proteins and/or protein fragments for use in the diagnosis of Kaposi's Sarcoma and associated herpesvirus (KSHV/HHV8).

It is a further aim to produce diagnostic kits, such as for example, an ELISA kit or competitive assay kit using these proteins and/or protein fragments.

It is a further aim to produce monoclonal or polyclonal antibodies to these proteins and/or protein fragments.

According to a first aspect of the present invention there is provided an immunogenic determinant comprising, consisting of or containing an amino acid sequence substantially homologous with the carboxyterminal end of an orf 65 protein.

According to a second aspect of the present invention there is provided an immunogenic determinant comprising, consisting of or containing an amino acid sequence:

ADRVSAASAY DAGTFTVPSR PGPASGTTPG GQDSLGVSGS SITTLSSGPH SLSPASDILT TLSSTTETAA PAVADARKPP SGKKK

According to a third aspect of the present invention there is a method of screening for an infection with KSHV and/or HHV8 which method utilises an immunogenic determinant of the invention.

According to a fourth aspect of the present invention there is provided an immunogenic determinant derived from the carboxyterminal end of an orf 65 protein for use in a vaccine or as a diagnostic tool against KSHV or HHV8.

According to a fifth aspect of the present invention there is provided an immuno-assay kit comprising an immunogenic determinant of the invention.

Preferably the kit is in the form of an ELISA or competitive assay kit.

According to a further aspect of the present invention there is provided an antibody directed to an immunogenic determinant of the invention.

Preferably the antibody is a monoclonal antibody.

The invention will now be further described, by way of example only, with reference to the following methodology and test data.

Methods

Immunoreactive capsid-related proteins of KSHV/HHV 8 were selected by expressing them as recombinant proteins in E. coli and testing their reactivity with patient sera in Western blot assays. One of these recombinants, encoded by open reading frame (orf) 65 was used, to develop an immuno assay, namely a diagnostic ELISA and sera from HIV-infected individuals with KS, from classic HIV-uninfected KS patients, from other HIV risk groups and blood donors was tested. The antibody response to this capsid-related protein was compared to the response to HHV 8 latent antigen(s) as measured in an immunofluorescence assay (IFA).

Expression of recombinant HHV 8 proteins

Open reading frames (orf) 52 and 65 (or segments) were amplified by PCR, fused to the carboxyterminal end of mouse dihydrofolate reductase (DHFR), and placed under the control of a T7 promoter using a commercially available expression vector, pQE 42 (Qiagen, Hilden, Germany). This vector also provides a histidine tag at the aminoterminal end of the fusion protein.

To generate construct orf 65.1 (amino acids 2 - 170 of orf 65), the primers GAG AGA GAT CTG TTC CAA CTT TAA GGT GAG AGA C and TCT GCA TGC CGG TTG TCC AAT CGT TGC CTA were used. These primers generate a 589 bp fragment which was cloned using the BglII and SphI sites introduced during the PCR.

Construct orf 65.2 (amino acids 86 - 170) was assembled in a similar manner using primers AGA GAG ATC TGT GCT GAC CGA GTT TCC GCG GCG and TCT GCA TGC CGG TTG TCC AAT CGT TGC CTA.

The orf 65.2 primers comprise respectively:

1) A stuffer sequence AGAG, a Bgl II restriction site AGATCT, GT and the coding sequence for the aminoacids ADRVSAA, and

2) A stuffer sequence TCT, a SphI restriction site GCATGC and a

sequence CGG TTG TCC AAT CGT TGC upstream of the CTA stop codon (amino acid number 171).

The carboxyterminal region of orf 65 (aa 86 - 170) is slightly less homologous (21% aa identity) to the equivalent region in EBV BFRF3 than the aminoterminal half (31% aa identity).

Recombinant proteins were purified by affinity chromatoraphy on Ni-NTA resin as described by the manufacturer (Qiagen, Hilden, Germany).

ELISA with orf 65.2 protein

ELISA plates (Immulon 4; Dynatech) were coated with 100 μl of purified orf 65.2 protein (approximately 5 μg/ml) in 0.1 M $NaHCO_3$, pH 8.5 for 16 h at room temperature. A conventional ELISA protocol was employed using PBS, 0.1% Tween 20 (PBS-T) for washes, 5% dried skimmed milk in PBS-T (blocking buffer) to saturate plates and to dilute patient sera (1:100), and an alkaline phosphatase (AP) conjugated affinity-purified goat anti-human IgG (Seralab), diluted 1:1000 in blocking buffer containing 1% goat serum, followed by 0.1 mg/ml of nitrophenyl phosphate (Sigma, St. Louis, USA) in glycine buffer (0.1 M glycine, 1 mM $MgCl_2$, 1 mM ZnCl, pH 10.4) as substrate. The colorimetric reaction was stopped after 1 hour at 37 °C with 50 μl of 3 M Na-hydroxide and read spectrophotometrically at 405 nm.

Immunofluorescence assay (IFA) for HHV 8 antibodies

This was carried out on a body-cavity related B-cell lymphoma cell line latently infected with HHV 8 (BCP-1), as described in (Gao, S.J., Kingsley, L., Li, M. et al. Seroprevalence of KSHV antibodies among North Americans, Italians, and Ugandans with and without Kaposi's sarcoma. *Nature Medicine* 1996; 2: 925 - 928). This assay detects antibodies to latent antigen(s) of HHV 8.

Patient sera

The following sera was analysed:

78 sera from HIV infected gay men attending an HIV clinic, of whom 57 had KS and 21 had AIDS in the absence of KS;

10 sera from HIV-infected Ugandan KS patients and 10 sera from Ugandan HIV-infected individuals without KS;

18 sera from classic Greek KS patients and 26 sera from their age and sex matched controls, obtained from an ongoing case control study in Athens, Greece (A.H.);

84 sera from haemophiliac patients, of whom 28 were infected with HIV;

63 sera from intravenous drug users in Edinburgh, of whom 38 were infected with HIV; 174 sera from UK blood donors; and

20 sera from US blood donors.

Statistical analysis

The concordance between IFA and ELISA was evaluated by calculating a κ statistic as described in (Fleiss, J.L. Statistical Methods for Rates and Proportions, 1981; 2nd edition).

Results

Selection of orfs and expression of recombinant antigens

Fig. 1 shows a Western Blot with recombinant orf 65 and orf 52, where

1 = orf52

2 = compete orf 65

3 = orf 65 aa 86 - 170

A = AIDS KS serum

B = blood donor system

C = UK AIDS KS serum (orf 65 ELISA+/IFA-/orf 65 WB+)

D = UK blood donor (orf 65 ELISA+/IFA-/orf 65 WB+)

E = Greek control patient (orf 65 ELISA+/IFA-/orf 65 WB+)

F = high titre EBV VCA serum (orf 65 ELISA+/orf 65 WB+)

G = UK blood donor (orf 65 ELISA+/IFA-/WB-)

Referring to Fig. 1 it will be seen that whilst both recombinant orf 65 proteins reacted well with most sera from KS patients (panel A, lanes 2,3), the recombinant orf 52 protein showed no significant reactivity (panel A, lane 1). Both recombinant orf 65.1 and orf 65.2 reacted with the same number of patient sera on Western blots. The carboxyterminal regions of orf 65 and BFRF3 share only 18 evenly distributed amino acids (21% identity) which makes antibody cross-reactivity between them unlikely.

- *ELISA with recombinant orf 65*

In the light of the aforementioned results the recombinant orf 65.2 was used as an antigen in a diagnostic ELISA.

Fig. 2 shows an example of the ELISA results obtained with recombinant orf 65 protein on a selection of human sera.

The results of 21 serum samples from the patient groups 1 to 5 were tested by ELISA at a 1:100 dilution. Patient groups 1 to 5 are as follows:

1: individuals with AIDS-associated KS;

2: AIDS patients without KS;

3: UK blood donors;

4: intravenous drug users:

5: haemophiliacs.

The thin line represents the "cut-off" value (0.16) determined as discussed in the text.

Among the AID-related KS patients many reactive samples were detected whilst among haemophiliacs, intravenous drug users (IVDU), and healthy blood donors only a few reactive samples were detected. To distinguish between reactive and unreactive sera, the mean of O.D. values of healthy blood donor sera (with the exception of the three strongly reactive sera shown in Fig. 2) plus three standard deviations as the "cut-off" value was used. To test blinded sera, the mean plus five standard deviations of a set of 10 negative controls to determine the "cut-off" value was used, and also included 2 sera which gave reactivities close to the cut-off, 2 low positive and

1 high positive sera, to ensure inter-assay comparability.

Using these criteria antibodies to orf 65 in 46 of 57 (80.7 %) UK or US AIDS-KS sera, 8 of 10 (80.0%) Ugandan AIDS-KS sera, and 17 out of 18 (94.4 %) of HIV-negative classic KS sera were detected as shown in Table 1 below:

Table 1: Antibodies to orf 65 protein of KSHV/HHV 8 in different HIV risk groups and controls

Risk groups	positive/tested	% positive
AIDS-KS		
UK/US	46/57	80.7
Uganda	8/10	80.0
Classic Greek KS	17/18	94.4
HIV-infected gay men		
without KS	5/16	31.3
Haemophiliacs	1/84	1.2
HIV infected	0/28	0.0
HIV uninfected	1/56	1.7
IVDU	2/63	3.2
HIV infected	2/38	5.3
HIV uninfected	0/25	0.0
Blood donors*		
UK	3/174	1.7
USA	1/20	5.0
Greek age/sex matched controls**	3/26	11.5
Ugandan controls	7/10	70.0

*Confirmed by Western blot on recombinant orf 65.1 and orf 65.2 (fig.1). 11 blood donor sera were initially reactive in the orf 65.2 ELISA

**confirmed by Western blot or IFA

Among gay men with AIDS, but no KS, antibodies in 5 of 16 cases were detected (31.3 %). 18/18 ELISA - reactive sera from individuals with AIDS-KS or AIDS in the absence of KS were also reactive by Western blot on recombinant orf 65.1 and 65.2 antigen (fig.1).

In contrast, antibodies to orf 65 were only detected infrequently in other risk groups for HIV transmission: 1/84 haemophiliac patients (1.2%) and 2/63 intravenous drug users (IVDU) (3.2 %) had antibodies to HHV 8 orf 65 (table 1). Among UK blood donors, 11/169 sera were reactive by ELISA. Of these, 3 sera were confirmed as positive by Western blotting using both recombinant orf 65.1 and orf 65.2 antigens (Fig.1), suggesting a prevalence of KSHV/HHV 8 infection in UK blood donors of at least 1.6 % table 1). 3 of 26 Greek control sera were reactive by ELISA, obtained from individuals who had been matched to the 18 classical KS cases by age and sex (table 1). All 3 ELISA reactive Greek control sera were also positive by WB and/or IFA, amounting to a seroprevalence of KSHV/HHV 8 in Greek elderly individuals of 11.5 %. In contrast, infection with KSHV/HHV 8 seems to be more widespread in Uganda, as 7/10 (70%) control samples had antibodies to KSHV/HHV 8 orf 65. Blinded sequential sera from 14 KS patients (2 - 4 sera from each individual) were also tested and concordant results were obtained. Only one individual was obtained in whose last (of three) serum sample antibodies were no longer detected to the orf 65 encoded protein.

Concordance of KSHV/HHV 8 antibody detection by orf 65 ELISA and immunofluorescence

The detection of antibodies to orf 65 and to latent KSHV/HHV 8 antigens was compared by testing sera from KS patients, individuals at risk for KS (gay men with AIDS, but without KS; Ugandan HIV-infected individuals), and UK and US blood donors in the orf 65 ELISA and immunofluorescence (IFA) on BCP-1 cells. These results ware shown in table 2 below:

Table 2

Comparison of antibody reactivity to orf 65 protein and to latent antigen(s)

	Reactivity to KSHV/HHV 8 latent antigen (IFA)					
	KS*		HIV-infection: no KS**		UK/US blood donors*** and Greek controls	
	+	-	+	-	+	-
Reactivity to KSHV/HHV 8 orf 65 (ELISA/WB) +	62	7	7	5	2	4
-	9	4	3	11	5	196
	$\kappa = 0.21$ $p = 0.023$ (1-tailed)		$\kappa = 0.37$ $p = 0.026$ (1-tailed)		$\kappa = 0.29$ $p = 0.000019$ (1-tailed)	

*includes UK/US and Ugandan AIDS KS cases

**includes HIV-infected gay men with AIDS, but without KS, and HIV-infected Ugandan subjects without KS

***includes Western blot confirmed blood donor results as discussed in the text.

There was good agreement between the two assays. 89.5% of all sera (282/315) showed a concordant result. 71 of 88 (80.7 %) IF-positive sera were also positive by ELISA and 71 of 87 (81.6 %) ELISA positive sera were also reactive in immunofluorescence. Among the 14 AIDS - related KS patients from whom sequential sera were available, 3 individuals who consistently lacked antibodies to the orf 65 - encoded protein despite being (on at least one occasion) reactive by IFA were observed, suggesting that the lack of orf 65 reactivity can be a stable phenomenon. 4 individuals were also observed with AIDS-related KS who were negative in both assays. The 3 ELISA-reactive, WB-confirmed UK blood donor sera were negative by IFA. Among the Ugandan samples tested, the concordance rate was 14/20 (70.0%), with 12/15 IFA-reactive samples being also ELISA reactive (80.0%), and 12/15 ELISA - reactive samples being also positive in IFA (80.0 %). This suggests that the high rate of KSHV/HHV 8 infection among HIV-infected Ugandans found by ELISA is genuine, and not due to non-specific reactivity.

Findings

The KSHV/HHV 8 orf 65 protein is recognised by the majority of sera from KS patients and its dominant immunogenic region is located within the carboxyterminal amino acids. This region shows only little homology (21% amino acid identity) with the corresponding region in the EBV genome and is thus likely to represent a specific serological antigen. By ELISA, the applicants detected antibodies to this domain in 46 of 57 (80.7%) UK AIDS-KS sera, 8 of 10 (80.0%) Ugandan AIDS-KS sera, 17 out of 18 (94.4 %) of HIV-negative classic KS sera, 1 out of 84 (1.2 %) of haemophiliac sera, and 2 of 63 (3.2 %) of IV drug user sera. 3 (of 174; 1.7%) ELISA reactive UK blood donor sera were confirmed by Western blot (WB) on recombinant proteins. Antibody reactivity to the orf 65 encoded protein and to latent antigen(s), measured by immunofluorescence, was concordant in 89.5% of all sera tested in both assays. A few sera, including four AIDS-KS sera, were unreactive in both assays. The three ELISA- and WB-positive blood donor sera

were unreactive with latent KSHV/HHV 8 antigen(s) in immunofluorescence.

Interpretation

The distribution of antibodies to both a capsid-related recombinant protein and latent antigen(s) of KSHV/HHV 8 strongly supports the view that infection with this virus is largely confined to individuals with, or at increased risk for, KS. However, infection with KSHV/HHV 8 does occur rarely in the general UK and US population and is more common in Uganda. Measured on their own, antibodies to neither latent antigen(s), nor to the orf 65 encoded capsid protein, detect all cases of KSHV/HHV 8 infection, and a combination of these antigens will be preferred for accurate screening assays.
The results demonstrate the recombinant HHV 8 protein is recognised by the majority of sera from KS patients and is therefore useful for the serological diagnosis of infection with KSHV/HHV 8.

Orf 65 encodes a protein which, based on its positional and (low) sequence homology with EBV BFRF3 is likely to be a capsid protein and is therefore expressed during the lytic cycle of viral replication. Because of its predicted low molecular weight (about 18 kd) it is not identical with a previously described 40 kd lytic-cycle HHV 8 protein, recognised by about 67% of patient sera, or the 27 kd and 60 kd lytic-cycle proteins which react with only a small proportion of KS sera. As in the case of EBV BFRF3, the immunogenic determinants in the orf 65 -encoded protein are located in its carboxyterminal half which shares only 21% of its amino acids with the corresponding BFRF3 segment. This region (aa 86 - 170) is therefore a good candidate for a specific serological antigen. Latently infected body-cavity related B-cell lymphoma cell lines (BCBL) express at least one latent antigen which has been reported to react (in IFA) with about 90 % of sera from AIDS-related KS patients and whose identity is presently unknown. A high molecular weight immunoreactive doublet antigen which is localised to the nucleus has also been reported but it is unknown whether this is the antigen being assayed in the BCP -1 IFA assay.

Using an ELISA based on recombinant orf 65.2 protein (aa 86 - 170 of orf 65), the applicant has found that 80.7 % of AIDS-KS sera, and 94.4 % of HIV-negative 'classical' KS sera, contain antibodies to this HHV 8 protein. In contrast, antibodies to orf 65 are only rarely found in haemophiliacs and IV drug users who have a low risk of developing KS, even after infection with HIV. Thus antibodies to both latent and capsid protein of KSHV/HHV 8 are rare in individuals at low risk for KS. In analogy to EBV, where antibodies to VCA (viral capsid antigen) and EBNA (EBV latent antigen) persist after infection with EBV, it is thus likely that the antibody response to both latent and capsid proteins of KSHV/HHV 8 is due to infection with, rather than reactivation of, KSHV/HHV 8. Thus serological data support the conclusion, that infection with HHV 8 is not common in the general UK and US population. It was found that the sensitivity of both the orf 65 ELISA and IFA were comparable and that a similar number of sera was missed by either assay alone. The applicant detected 3 (of 174; 1.6%) UK blood donor sera with Western blot confirmed antibody reactivity to orf 65 which scored negative in the immunofluorescence assay. In view of the low homology between orf 65 and EBV BFRF3 it is very unlikely that these sera could have contained antibodies to EBV BFRF3 which cross-reacted with the orf 65.2 recombinant protein. Of 10 sera with high titre EBV VCA antibodies none reacted with both recombinant orf 65 proteins. However, taken together with the recently reported small number of IFA-reactive US blood donor sera (, these findings suggest a low percentage of KSHV/HHV 8 - infected individuals in the general UK and US populations. The results with a capsid-related antigen also confirm that KSHV/HHV 8 infection is more common in some parts of Africa (e.g. Uganda) than in the UK or US but its exact prevalence in different geographical areas remains to be established, most likely by a combination of tests measuring antibodies to both lytic and latent antigens, as illustrated here.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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(A) APPLICATION NUMBER: GB 9618890.9
(B) FILING DATE: 10-SEP-1996

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 85 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Ala Asp Arg Val Ser Ala Ala Ser Ala Tyr Asp Ala Gly Thr Phe Thr
1 5 10 15

Val Pro Ser Arg Pro Gly Pro Ala Ser Gly Thr Thr Pro Gly Gly Gln
20 25 30

Asp Ser Leu Gly Val Ser Gly Ser Ser Ile Thr Thr Leu Ser Ser Gly

35 40 45

Pro His Ser Leu Ser Pro Ala Ser Asp Ile Leu Thr Thr Leu Ser Ser
50 55 60

Thr Thr Glu Thr Ala Ala Pro Ala Val Ala Asp Ala Arg Lys Pro Pro
65 70 75 80

Ser Gly Lys Lys Lys
85

CLAIMS

1. An immunogenic determinant comprising, consisting of or containing an amino acid sequence substantially homologous with the carboxyterminal end of an orf 65 protein.

2. An immunogenic determinant comprising, consisting of or containing an amino acid sequence:

ADRVSAASAY DAGTFTVPSR PGPASGTTPG GQDSLGVSGS SITTLSSGPH SLSPASDILT TLSSTTETAA PAVADARKPP SGKKK

3. A method of screening for a KSHV and/or HHV8 infection which method utilises an immunogenic determinant as claimed in claim 1 or 2.

4. An immunogenic determinant as claimed in claim 1 or 2 for use in a vaccine or as a diagnostic tool against KSHV or HHV8.

5. An immuno-assay kit comprising an immunogenic determinant as claimed in claim 1 or 2.

6. An immuno-assay kit as claimed in claim 5 which is an ELISA assay kit.

7. An immuno-assay kit as claimed in claim 5 which is a competitive assay kit.

8. An antibody directed to an immunogenic determinant as claimed in claim 1 or 2.

9. An antibody as claimed in claim 8 which is a monoclonal antibody.

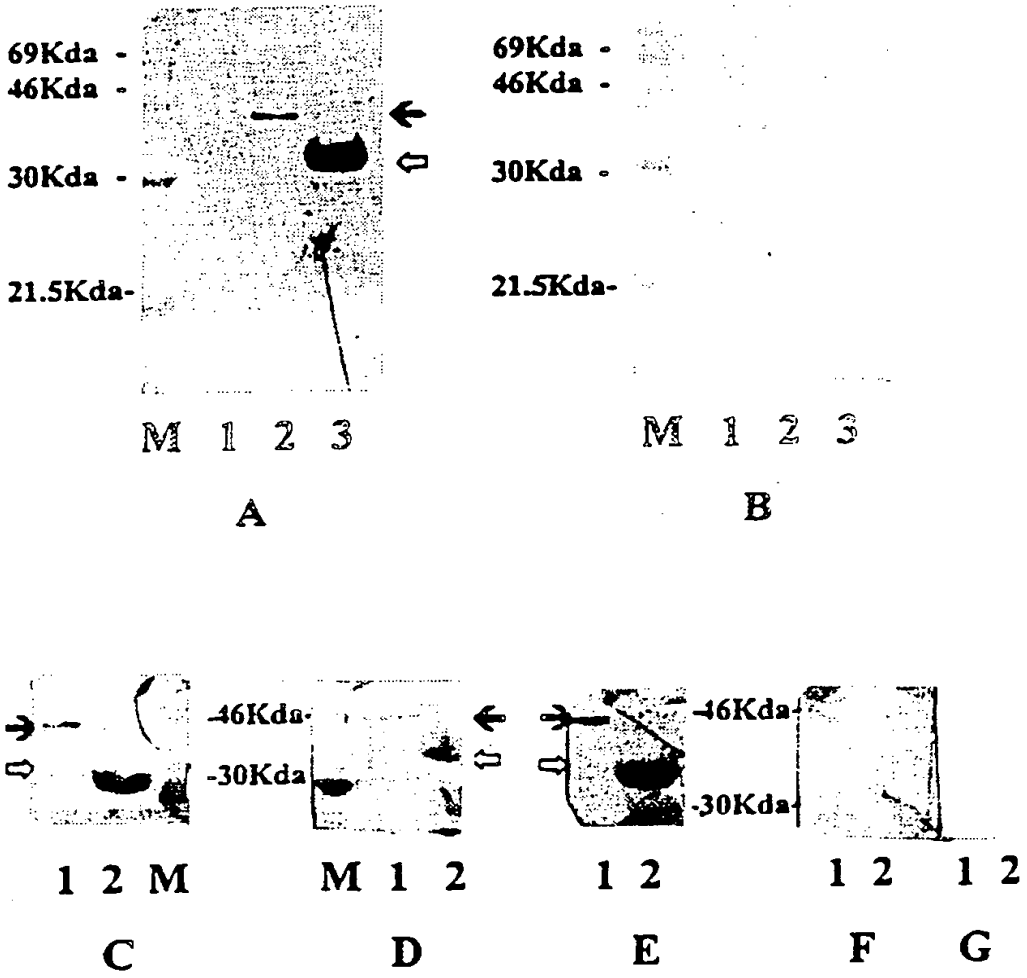
69Kda -
46Kda -
30Kda -
21.5Kda-
M 1 2 3
A
69Kda -
46Kda -
30Kda -
21.5Kda-
M 1 2 3
B
46Kda-
-30Kda
1 2 M
C
M 1 2
D
46Kda-
-30Kda
1 2
E
1 2
F
1 2
G

FIG. 1

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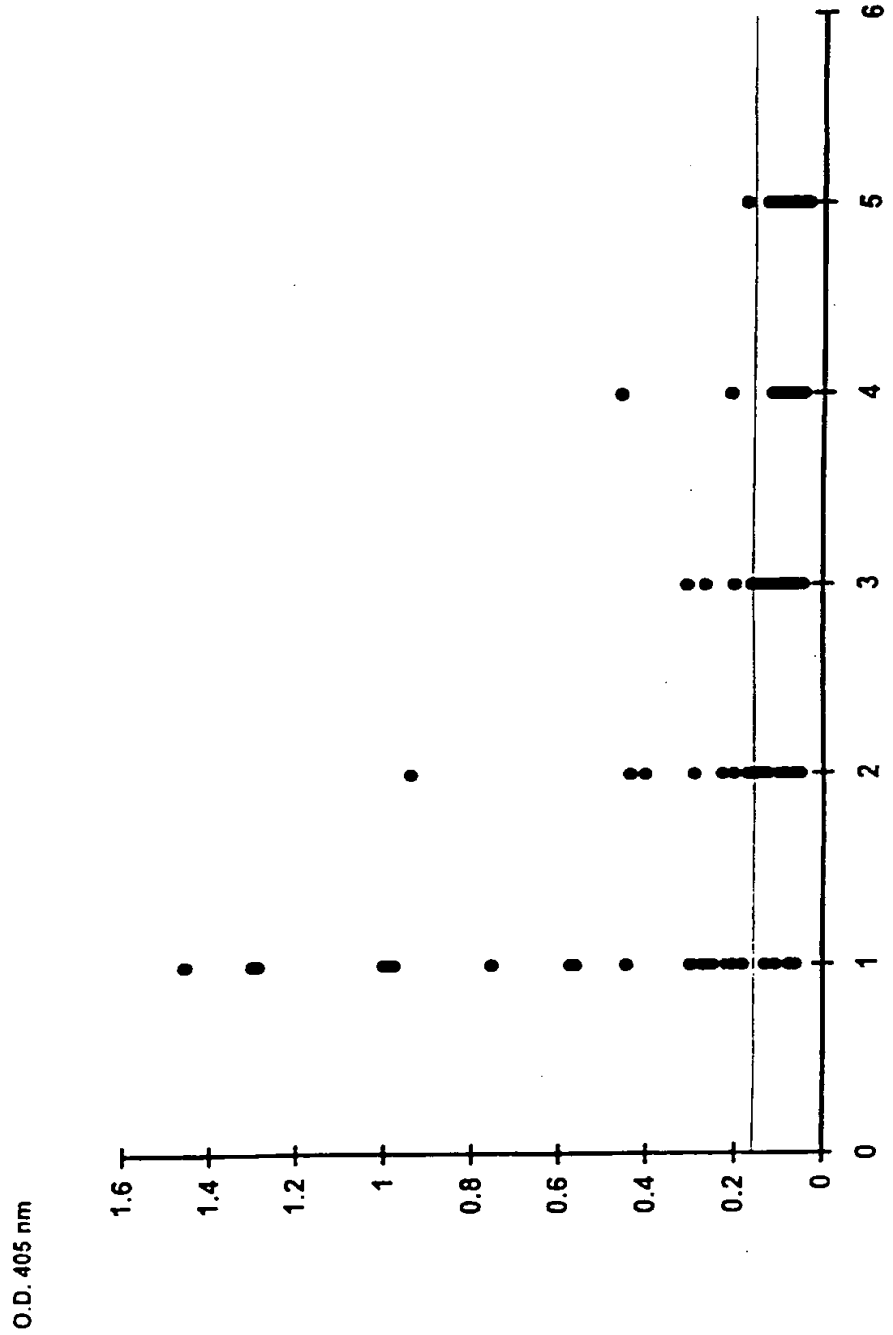

FIG. 2

TERNATIONAL SEARCH REPORT

ional Application No
PCT/GB 97/02487

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K14/03 A61K39/245

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	EMBL Database Accession number U75698 Created 23/12/1996 Kaposi's sarcoma-associated herpesvirus XP002050216	1-9
X	& P.CHANG ET AL.: "IDENTIFICATION OF HERPESVIRUS-LIKE DNA SEQUENCES IN AIDS-ASSOCIATED KAPOSI'S SARCOMA" SCIENCE, vol. 266, 16 December 1994, pages 1865-1869, XP000601301 see the whole document	1-9
X	WO 96 06159 A (UNIV COLUMBIA ;CHANG YUAN (US); MOORE PATRICK S (US)) 29 February 1996 see the whole document	1-9

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[X] Further documents are listed in the continuation of box C

[X] Patent family members are listed in annex

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

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Date of the actual completion of the international search
15 December 1997

Date of mailing of the international search report
15/01/1998

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Authorized officer
Cervigni, S

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
X	P.S. MOORE ET AL.: "Primary characterisation of a herpesvirus agent associated with kaposi's sarcoma" J. VIROLOGY, vol. 70, no. 1, January 1996, pages 549-558, XP002050060 cited in the application see the whole document	1-9
X,P	G.R. SIMPSON ET AL.: "Prevalence of Kaposi's sarcoma associated herpesvirus infection measured by antibodies to recombinant capsid protein and latent immunofluorescence antigen" THE LANCET, vol. 348, 26 October 1996, pages 1133-1138, XP002050059 see the whole document	1-9
X,P	RUSSO J J ET AL: "NUCLEOTIDE SEQUENCE OF THE KAPOSI SARCOMA-ASSOCIATED HERPESVIRUS (HHV8)" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 93, no. 25, 10 December 1996, pages 14862-14867, XP000645332 see figure 1; table 1 see the whole document	1-9
A	W. ZHONG ET AL: "RESTRICTED EXPRESSION OF KAPOSI SARCOMA-ASSOCIATED HERPESVIRUS GENES IN KAPOSI SARCOMA" PROC. NATL- ACAD. SCI. USA, vol. 93, June 1996, pages 6641-6646, XP002050061 see the whole document	

...ERNATIONAL SEARCH REPORT

...formation on patent family members

...tional Application No
PC...I/GB 97/02487

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9606159 A	29-02-96	AU 3321095 A	14-03-96
		AU 4367096 A	17-06-96
		CA 2196892 A	29-02-96
		EP 0804547 A	05-11-97
		WO 9615779 A	30-05-96